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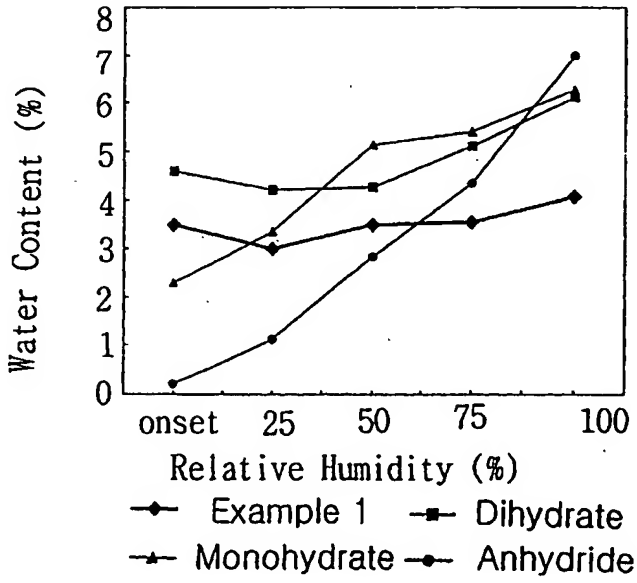
(54) Title: CLATHRATE OF AZITHROMYCIN HYDRATE WITH 1,2-PROPYLENEGLYCOL, METHOD FOR THE MANUFACTURE THEREOF AND PHARMACEUTICAL COMPOSITION COMPRISING SAME



(57) Abstract: A clathrate of azithromycin hydrate with 1,2-propyleneglycol is much less hygroscopic than azithromycin hydrate or crystals known in the art, therefore, it can be useful for the preparation of a medicine for treating various microbial infections.

WO 02/085898 A1

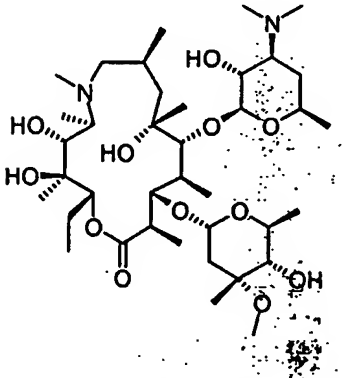
CLATHRATE OF AZITHROMYCIN HYDRATE WITH 1,2-PROPYLENEGLYCOL, METHOD FOR THE MANUFACTURE THEREOF AND PHARMACEUTICAL COMPOSITION COMPRISING SAME

Field of the Invention

This invention relates to a novel clathrate of azithromycin hydrate with 1,2-propyleneglycol, a process for its manufacture, and a pharmaceutical composition containing the clathrate.

Background of the Invention

Azithromycin, 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (N-methyl-11-aza-10-deoxo-10-dehydroerythromycin A: IUPAC) of formula (II) disclosed in U.S. Patent Nos. 4,517,358 and 4,474,768, is an azalide-type semi-synthetic macrolide antibiotic, useful for treating bronchial infection, sexual contact infection and dermatological infection (*See* Kirste and Sides; *Antimicrob. Agents Chemother.*, **33**, 1419(1989)).



(II)

Azithromycin is known to exist in three forms, the anhydride, monohydrate and dihydrate forms. These forms have been identified by powder X-ray

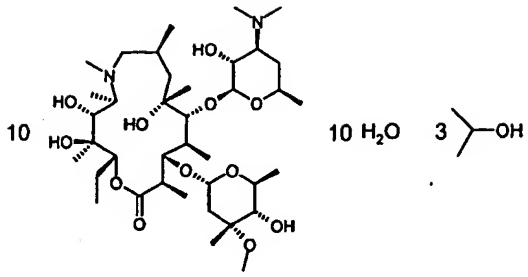
diffraction and differential scanning calorimetric studies.

Azithromycin anhydride, which is disclosed in U.S. Patent No. 4,517,359, is non-crystalline product and thus, its highly hygroscopic property is not suitable for pharmaceutical formulation.

Further, azithromycin monohydrate (mp. 136℃), as described in U.S. Patent No. 4,474,768 and WO Publication No. 89/00576, is crystalline but it has also hygroscopic property, making it difficult to maintain its water content at a constant level.

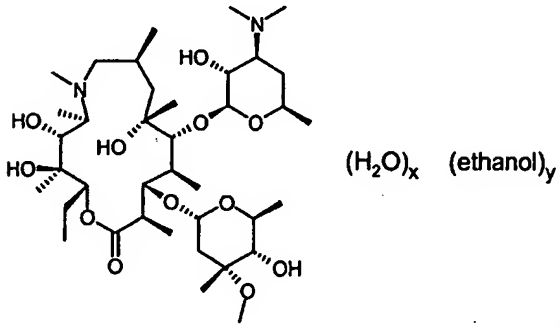
WO Publication No. 89/00576 discloses a process for preparing azithromycin dihydrate (mp. 126℃) from azithromycin monohydrate by recrystallizing from a mixture of tetrahydrofuran, water and a C_5~C_7 aliphatic hydrocarbon. Although the dihydrate is less hydroscopic than the monohydrate, the water content thereof must be carefully maintained during a vaccum drying step at a relatively low temperature. Such a water content controlling procedure is, however, not sufficient for removing the toxic aliphatic hydrocarbon solvent rigorously used in the recrystallization procedure. On the other hand, vacuum drying in higher temperature may result in formation of azithromycin dihydrate having undesirable water content.

Accordingly, many attempts have been made to develop a novel crystal or solvate form of azithromycin. For example, EP Publication No. 0,984,020 discloses a clathrate of azithromycin monohydrate with isopropanol of formula (III).



(III)

WO Publication No. 00/32203 discloses an ethanol solvate of azithromycin hydrate of formula (IV).



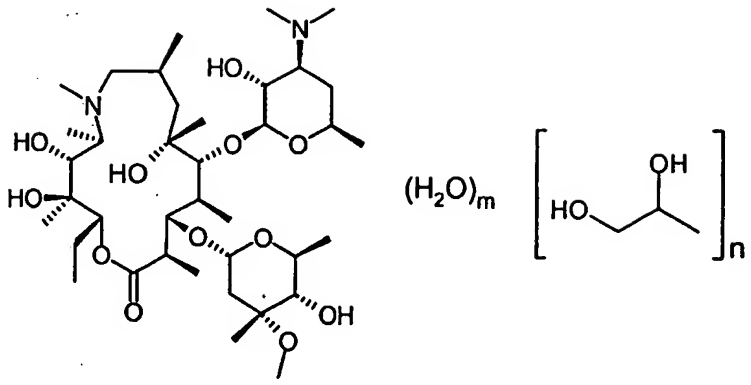
(IV)

However, there has existed a need to develop an improved crystal form of azithromycin crystal suitable for pharmaceutical applications.

Summary of the Invention

It is, therefore, an object of the present invention to provide a novel form of azithromycin, which can be useful for the preparation of a medicine for treating various microbial infections.

In accordance with the present invention, there is provided a novel clathrate of azithromycin hydrate with 1,2-propyleneglycol of formula (I):



(I)

wherein m ranges from 1 to 2 and n, from 0.20 to 0.40.

The present invention further provides a process for preparing the clathrate of formula (I), comprising the steps of: (1) dissolving azithromycin in acetone then adding 1,2-propyleneglycol and water thereto to obtain a crystalline product; and (2) filtering the crystals formed, washing the crystals with water and drying to produce the azithromycin clathrate crystals.

The present invention also provides a pharmaceutical composition for treating microbial infection, comprising the clathrate of formula (I) and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects and features of the present invention will become apparent from the following description of the invention, when taken in conjunction with the accompanying drawings, which respectively show:

Fig. 1: a powder X-ray diffraction spectrum of the compound of the present

invention;

Fig. 2: a powder X-ray diffraction spectrum of azithromycin monohydrate;

Fig. 3: a powder X-ray diffraction spectrum of azithromycin dihydrate ;

Fig. 4: a differential scanning calorimetric scan of the compound of the present invention;

Fig. 5: a differential scanning calorimetric scan of azithromycin monohydrate;

Fig. 6: a differential scanning calorimetric scan of azithromycin dihydrate;

Fig. 7: comparative hygroscopic properties of the compound of the present invention, azithromycin anhydride, monohydrate, and dihydrate.

Detailed Description of the Invention

The compound of formula (I) may be prepared by (1) dissolving azithromycin in a suitable amount of acetone, preferably 2 to 10㎖ of acetone per g of azithromycin, adding 1,2-propyleneglycol thereto in an amount of 0.25 to 2.5 ㎖ based on 1㎖ of acetone while maintaining at a temperature ranging from room temperature (R.T.) to the boiling point of acetone, adding water in an amount of 1 to 3㎖ per ㎖ of acetone, stirring the mixture for 30 minutes to 4 hours at a temperature ranging from 0℃ to room temperature, filtering precipitated crystals, washing the crystals with water and drying for 12 to 24 hours at a temperature ranging from 40℃ to 45℃.

The 1,2-propyleneglycol moiety of the inventive clathrate is essentially non-toxic (LD_{50}: 25㎖/kg, at oral administration in rat), and it can exist in the form of a racemate, an S-isomer, or an R-isomer.

The azithromycin being used in the preparation of the inventive clathrate may be anhydride, monohydrate, dihydrate, isopropanol clathrate, or ethanol solvate of azithromycin known in the art or a mixture thereof, and it can be prepared by any of the methods disclosed in U.S. Patent Nos. 4,517,359 and 4,474,768 and Korean Patent Application No. 2001-14659.

The novel clathrate compound of the present invention melts approximately at 130℃, shows in a DSC scan an endothermic peak at 150.8℃ and heat capacity of 104.42 J/g, as shown in Fig. 4. These thermal properties are completely different from those of the monohydrate form (endothermic peak 145.44℃; heat capacity: 137.37 J/g) or the dihydrate form (endothermic peak 142.72℃; heat capacity: 160.15 J/g), shown in Fig. 5 and Fig. 6, respectively.

The crystal structure of the clathrate compound of the present invention differs from those of the monohydrate and dihydrate form, as the powder X-ray diffraction patterns shown in Fig. 1, Fig. 2 and Fig. 3, respectively.

The water content of the inventive clathrate determined by a Karl-Fischer water analyzer ranges from 2.3 to 4.6%, preferably, from 3.0 to 4.0%, more preferably, from 3.1 to 3.7%, while its 1,2-propyleneglycol content determined with a gas chromatography or ^{1}H-NMR spectroscopy ranges

from 2.1 to 4.1%, preferably, from 2.4 to 3.8%.

The inventive clathrate of formula (I) preferably has an m value of 1.5±0.2 and an n value of 0.30±0.06.

The clathrate compound of the present invention is much less hygroscopic than azithromycin anhydride or azithromycin monohydrate, and its water content remains more or less constant when stored under a humid condition, unlike azithromycin dihydrate.

The clathrate compound of present invention can be used in formulating various pharmaceutical compositions for treating various microbial infection. Such a composition contains the inventive clathrate together with pharmaceutically acceptable excipients and carriers, which may be administrated orally, injectably, rectally, transdermally, bucally or nasally. Suitable forms for oral administration include tablets, compressed or coated pills, dragees, sachets of powder for reconstitution, hard or soft gelatin capsules, syrups and emulsions et al. Suitable forms for parenteral administration include aqueous or non-aqueous solution, emulsion, while for rectal administration suitable forms include suppositories with hydrophilic or hydrophobic vehicles. For topical application the invention provides ointments or aerosol formulations known in the art; for transdermal delivery, there are provided suitable delivery systems as known in the art. For nasal delivery there are provided suitable aerosol delivery systems known in the art.

This invention will be better understood from the Examples that follow.

However, the examples illustrate, but do not limit, the invention. Those skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

Example 1

100g of azithromycin anhydride was dissolved in 300㎖ of acetone and 100 ㎖ of 1,2-propylene glycol was added thereto. The solution was stirred for 10 minutes at R.T. and 500㎖ of water was added dropwise thereto to induce the precipitation of azithromycin crystals. The solution was stirred for 2 hours at R.T. and the precipitate was filtered, washed rigorously with water, and then dried at 40℃ for 20 hours to give 96g of a clathrate of azithromycin hydrate with 1,2-propyleneglycol.

m. p: 129 to 131℃,

The water content determined by a Karl Fischer water analyzer: 3.5 wt%,

The 1,2-propyleneglycol content determined with a gas chromatography: 3.3 wt%.

Example 2

20g of azithromycin monohydrate was dissolved in 100㎖ of acetone and 15 ㎖ of 1,2-propylene glycol was added thereto. The solution was stirred for 10 minutes at R.T. and 200㎖ of water was added dropwise thereto to induce the precipitation of azithromycin crystals. The solution was stirred for 2 hours at 0 to 5℃ and the precipitate was filtered, washed rigorously with water, and then dried at 40℃ for 20 hours to give 18.2g of a clathrate of azithromycin hydrate with 1,2-propyleneglycol.

m. p: 130 to 132℃,

The water content: 3.4 wt%,

The amount of 1,2-propyleneglycol: 3.2 wt%.

Example 3

20g of azithromycin monohydrate was dissolved in 120㎖ of acetone and 15 ㎖ of 1,2-propylene glycol was added thereto. The solution was stirred for 10 minutes at R.T. and 180㎖ of water was added dropwise thereto to induce the precipitation of azithromycin crystals. The solution was stirred for 3 hours at 0 to 5℃ and the precipitate was filtered, washed rigorously with water, and then dried at 40℃ for 20 hours to give 17.6g of a clathrate of azithromycin hydrate with 1,2-propyleneglycol.

m. p: 130 to 132℃,

The water content: 3.4 wt%,

The 1,2-propyleneglycol content: 3.5 wt%.

Test Example 1

The compound obtained in Example 1, azithromycin monohydrate and dihydrate obtained by the methods in accordance with U.S. Patent No. 5,869,629 were subjected to differential scanning calorimetric measurements (heat speed 10℃/minutes.). The inventive compound of Example 1 showed an endothermic peak at 150.8℃ and heat capacity of 104.42 J/g, as shown in Fig. 4. The azithromycin monohydrate, on the other hand, showed an endothermic peak at 145.44℃ and heat capacity of 137.37 J/g (Fig. 5), while azithromycin dihydrate, an endothermic peak at 142.72℃ and heat

capacity of 160.15 J/g (Fig. 6).

Further, the X-ray diffraction spectra of above three compounds are illustrated in Fig. 1, Fig. 2 and Fig. 3, respectively. The X-ray results summarized in Table 1 show that the compound of present invention has a crystal structure which is completely different from those of the known compounds.

Also, the hygroscopic properties of each of the compound obtained in Example 1 (1), azithromycin dihydrate (2), monohydrate (3), and anhydride (4) were determined by exposing each sample to a relative humidity of each 25%, 50%, 75% or 100% for 7 days and measuring the water content thereof by the Karl Fischer method. The result is shown in Table 2 and Fig. 7.

Table 1.

Radiation : Cu K-α1	Operation: 40kV/126mA
Divergence slit : 1°	Scan Mode: continuous
Scattering slit : 1°	Scan speed : 5°/min
Receiving slit: 0.15mm	Scan step: 0.02°

2 theta(°2Θ)	d-value(A)	I/Io(≥2)	2 theta(°2Θ)	d-value(A)	I/Io(≥2)
6.200	14.2437	3	18.300	4.8439	3
7.300	12.0996	5	18.500	4.7920	5
7.820	11.2962	32	19.040	4.6573	12
8.220	10.7474	2	19.660	4.5118	9
9.740	9.0733	100	19.980	4.4403	12
10.220	8.6482	2	20.400	4.3498	10
11.140	7.9360	29	20.860	4.2549	8
11.900	7.4308	7	21.740	4.0846	4
12.220	7.2369	6	22.320	3.9798	3
12.500	7.0754	22	22.640	3.9242	5
13.880	6.3749	12	23.220	3.8275	2
14.640	6.0456	16	23.540	3.7762	3
15.220	5.8165	12	23.960	3.7109	3
15.400	5.7490	12	24.520	3.6274	4
15.700	5.6398	6	24.720	3.5985	3
15.940	5.5554	6	25.260	3.5228	2
16.620	5.3296	6	25.500	3.4902	3
16.960	5.2235	10	26.200	3.3985	4
17.220	5.1452	9	28.440	3.1357	2
17.460	5.0750	11	31.080	2.8751	2
18.060	4.9078	2	33.600	2.6650	2

Table 2

	(1)	(2)	(3)	(4)
Onset	3.50	4.58(4.1)	2.30(3.2)	0.22
Relative humidity 100%	4.06	6.11(5.2)	6.29(7.2)	7.00
Relative humidity 75%	3.55	5.10(4.6)	5.41(6.6)	4.33
Relative humidity 50%	3.50	4.25(4.6)	5.13(5.6)	2.85
Relative humidity 25% (33%)	3.01	4.20(2.5)	3.35(2.3)	1.11
Calculated water content (%)	3.38[1)]	4.60	2.35	0.00
Found-Calculated (Difference,%)	-0.37~ +0.68	-0.4~ +1.51 (-2.1~ +0.6)	+1~+3.94 (-0.05~ +4.84)	+1.11~ +7.00
Range of Difference (%)	1.05	1.91(2.7)	3.94(4.9)	7.00

Note:

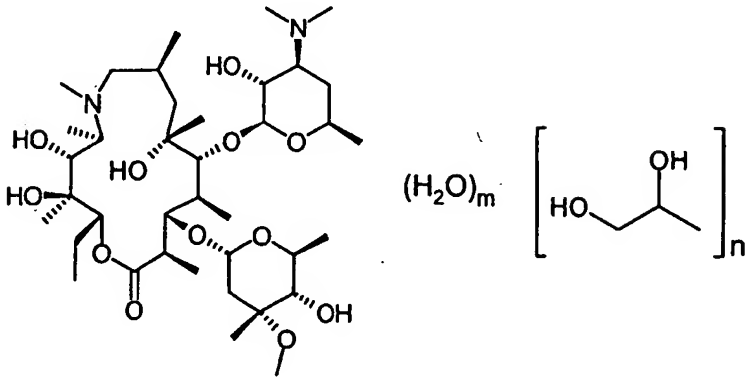
1) Calculated based on m=1.5 and n=0.30 in formula (I).
2) The numbers in parenthesis are values obtained after 3 days at the corresponding relative humidity.

Table 2 clearly shows that the novel clathrate compound of the present invention is much less hygroscopic than other compounds.

While the embodiments of the subject invention have been described and illustrated, it is obvious that various changes and modifications can be made therein without departing from the spirit of the present invention which should be limited only by the scope of the appended claims.

What is claimed is:

1. A clathrate compound of azithromycin hydrate with 1,2-propyleneglycol of formula (I):



(I)

wherein m ranges from 1 to 2 and n, from 0.20 to 0.40.

2. The compound of claim 1, wherein water content ranges from 2.3 to 4.6 % and 1,2-propyleneglycol content is between 1.9 and 3.8%.

3. A process for preparing the azithromycin clathrate compound of formula (I), comprising the steps of: (1) dissolving azithromycin in acetone and adding 1,2-propyleneglycol thereto to obtain a crystalline product; and (2) filtering the crystals formed, washing the crystals with water and drying to produce the azithromycin clathrate crystals.

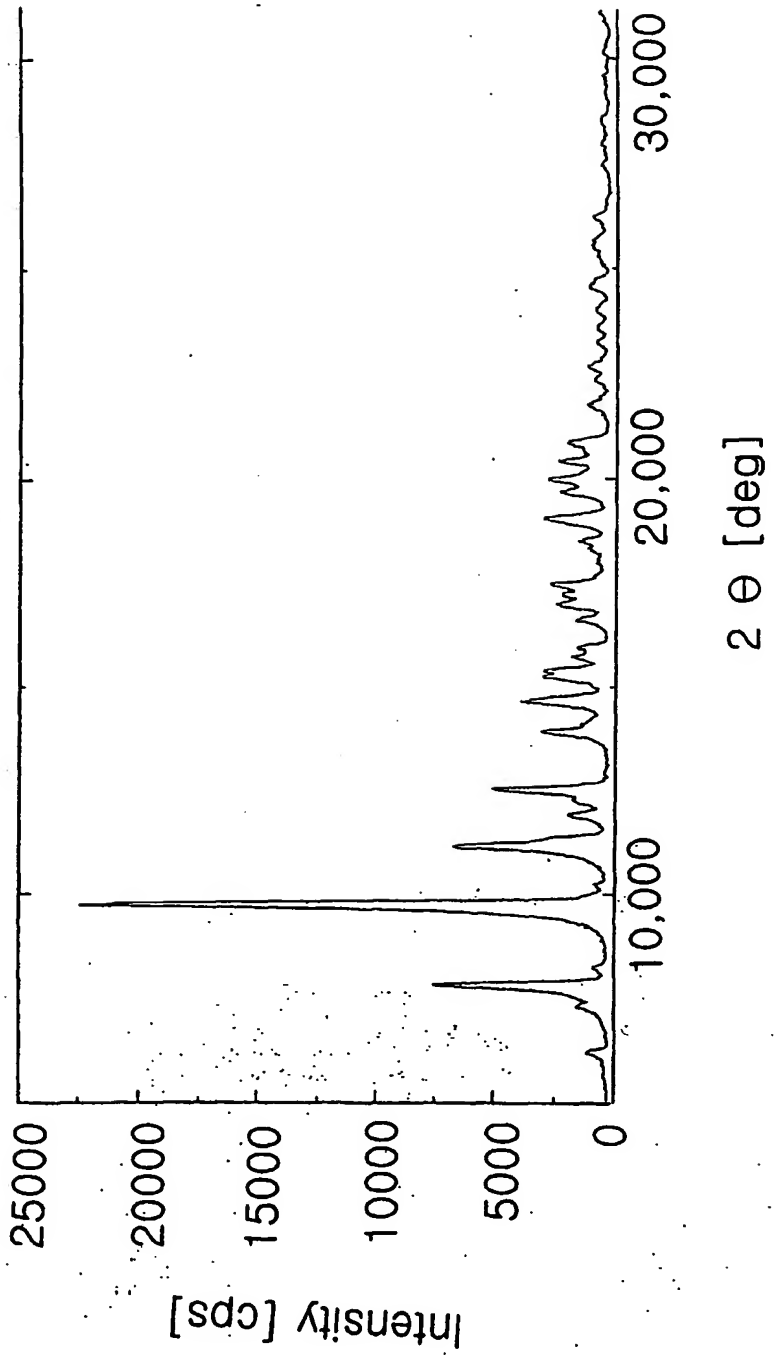
4. The process of claim 3, wherein 2 to 10㎖ of acetone is employed per g of azithromycin.

5. The process of claim 3, wherein 0.25 to 2.5㎖ of 1,2-propyleneglycol is used per ㎖ of acetone.

6. The process of claim 3, wherein 1 to 3㎖ of water is used per ㎖ of acetone.

7. A pharmaceutical composition for treating microbial infection, comprising the azithromycin clathrate compound of formula (I) and a pharmaceutically acceptable carrier.

FIG. 1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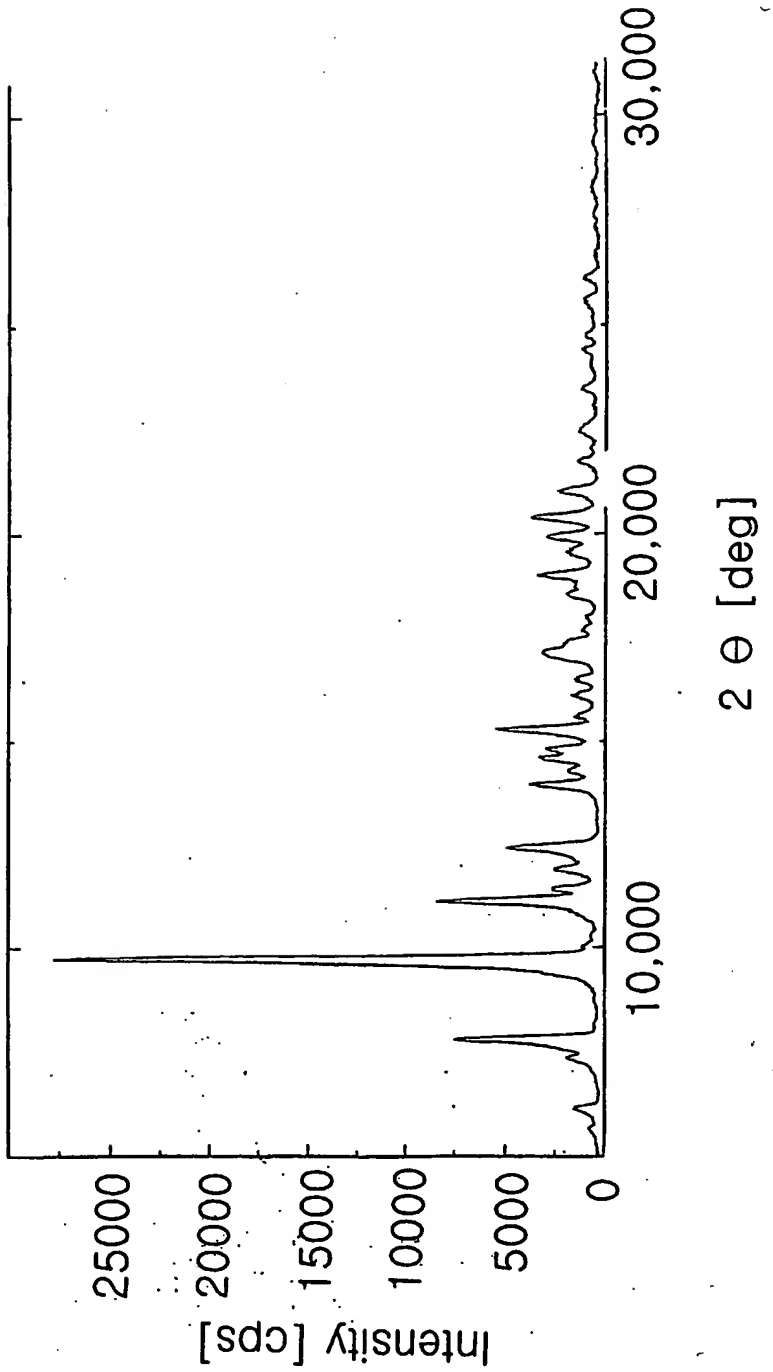
25000
20000
15000
10000
5000
0
10,000
20,000
30,000
2 θ [deg]

FIG. 2


Intensity [cps]
25000
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10,000
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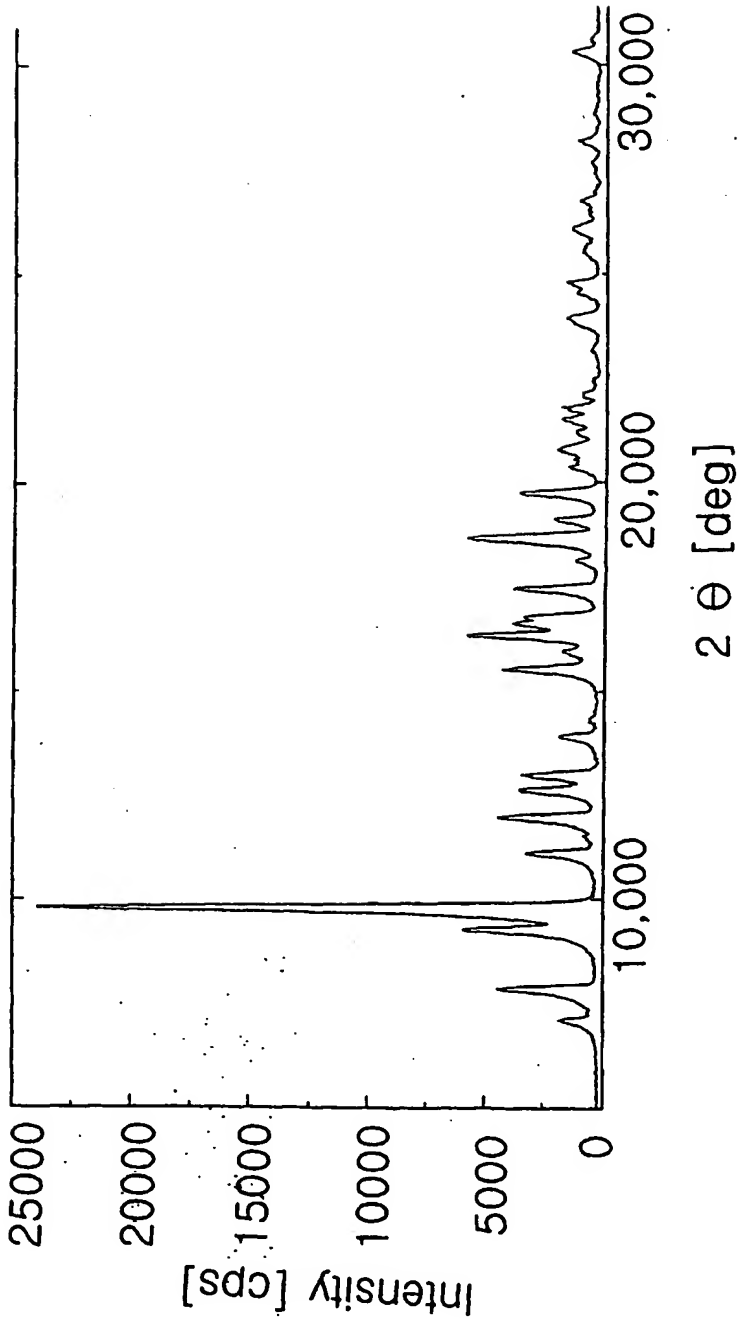
2 Θ [deg]

FIG. 3


Intensity [cps]
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15000
10000
5000
0
10,000
20,000
30,000
2 θ [deg]

FIG. 4

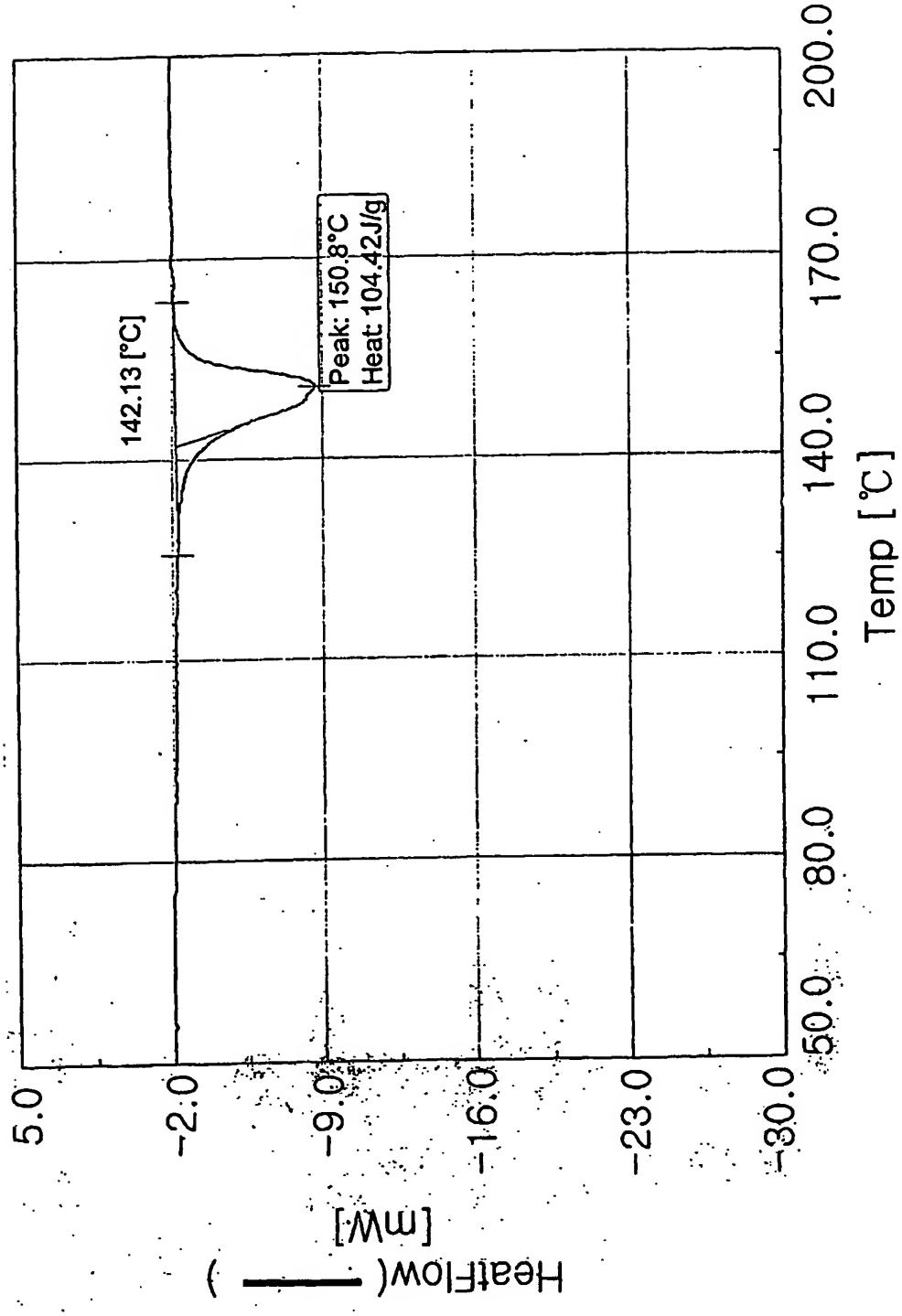

142.13 [°C]
Peak: 150.8°C
Heat: 104.42J/g
200.0
170.0
140.0
110.0
80.0
50.0
Temp [°C]
5.0
-2.0
-9.0
-16.0
-23.0
-30.0
HeatFlow(———)
[mW]

FIG. 5

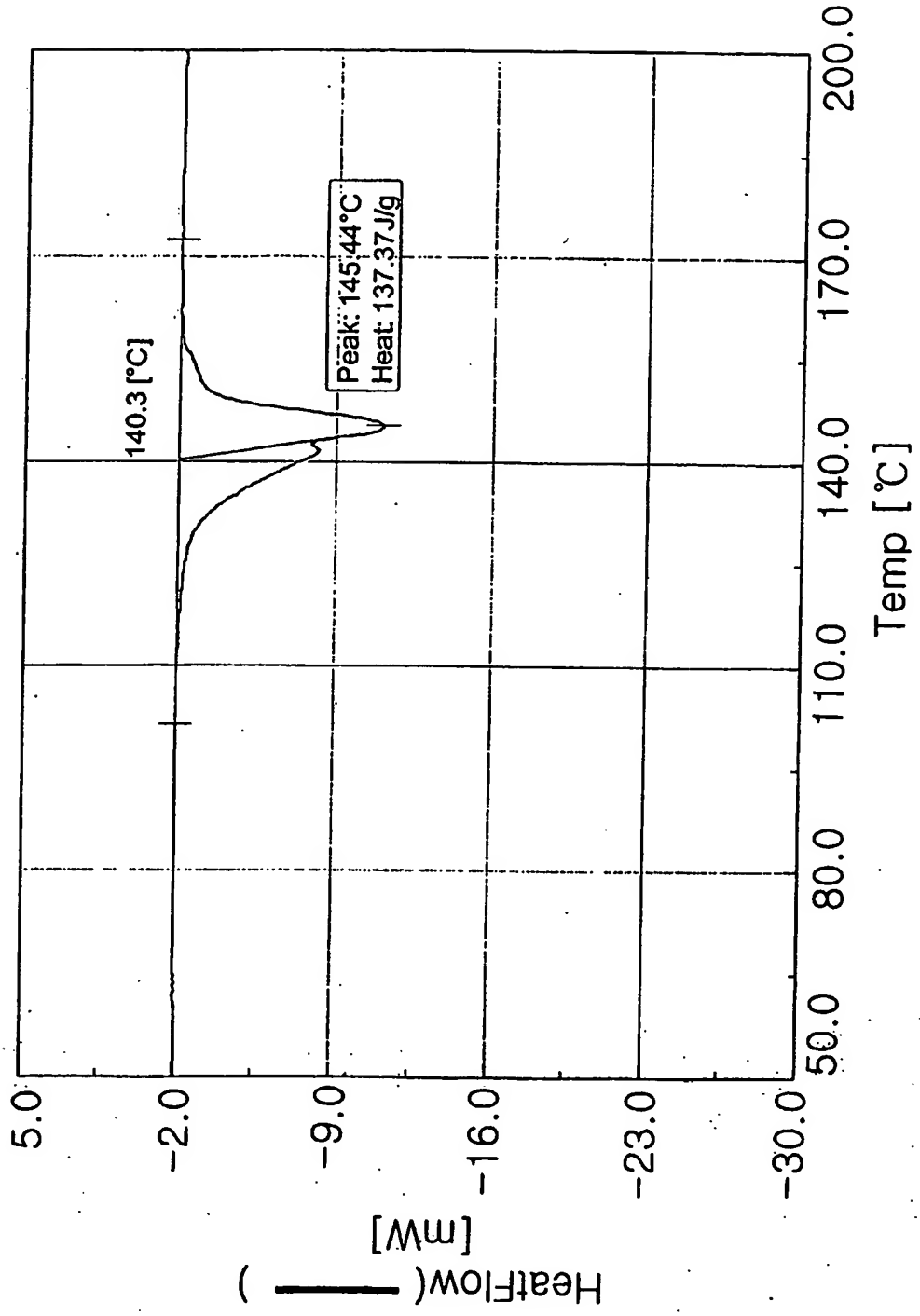
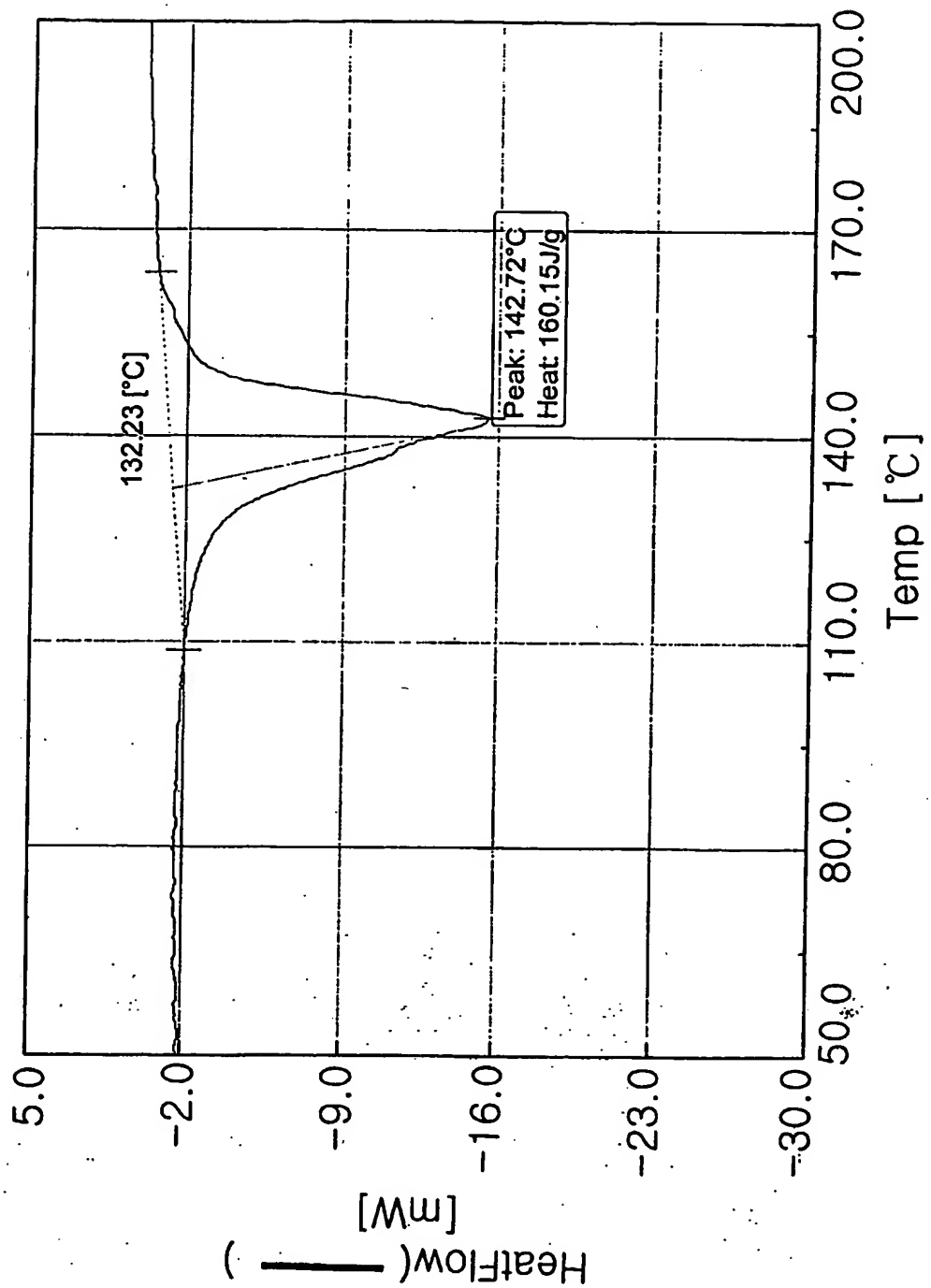

Peak: 145.44°C
Heat: 137.37J/g
140.3 [°C]
200.0
170.0
140.0
110.0
80.0
50.0
Temp [°C]
5.0
-2.0
-9.0
-16.0
-23.0
-30.0
HeatFlow(———)
[mW]

FIG. 6


Peak: 142.72°C
Heat: 160.15J/g
132.23 [°C]
Temp [°C]
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170.0
140.0
110.0
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HeatFlow( )
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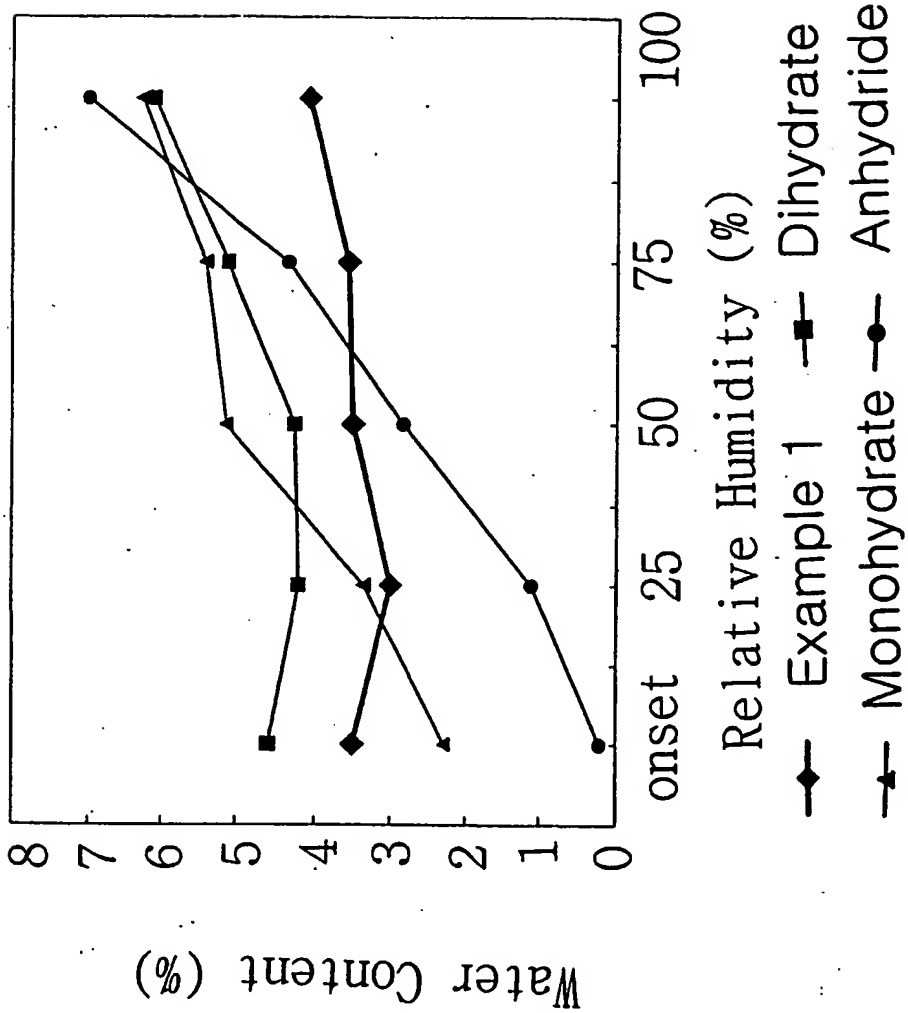
FIG. 7


Water Content (%)
0
1
2
3
4
5
6
7
8
onset
25
50
75
100
Relative Humidity (%)
Example 1
Dihydrate
Monohydrate
Anhydride

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00761

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07D 413/14, A61K 31/7028,

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC7 C07D, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/7766 A (Cadila Pharm. Ltd, India) 31. 01..2002 see abstract and claim	1-7
A	US 6,239,112 B (Merial, Inc., USA) 29. 05. 2001 See abstract	1-7
A	WO 00/57866 A (Insite Vision Incorporated, USA) 05.10.2000 see abstract	1-7
A	US 5,958,888 A (Merial, Inc.,USA) 28. 09.1999 see abstract and claim	1-7
A	BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS, 1998, 8 (5), 549-54 " Synthesis and antibacterial activity of O-methyl derivatives of azalide antibiotics : I. 4", 11 and 12-OMe derivatives via direct methylation" Waddell S. T. et al. see entire document	1-7
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☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
08 AUGUST 2002 (08.08.2002)	12 AUGUST 2002 (12.08.2002)

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INTERNATIONAL SEARCH REPORT

International application No.
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